PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application	:	Daniel R. Dietrich, et al.
Application No.	:	10/070,302
Filed	:	May 1, 2002
Confirmation No.	:	2837
For	:	CONGENER INDEPENDENT DETECTION OF MICROSYSTIN AND NODULARIN CONGENERS
Examiner	:	Mary Ceperley
Attorney's Docket	:	MBP-010XX

TC Art Unit: 1641

* *

I hereby certify that this correspondence is being sent via facsimile to Examiner Ceperley, TC Art Unit 1641, Fax No. (703) 872 9306, on 4-13-4.

By: _____
Charles L. Gagnebin III
Registration No. 25,467
Attorney for Applicant(s)

* *

DECLARATION OF DANIEL R. DIETRICH
UNDER 37 C.F.R. §1.132

Via Facsimile
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Daniel R. Dietrich, a citizen of Switzerland, residing at 8566 Neuwilen, Switzerland, declare the following:

1. I received my **(please provide your educational background and present position)**. see CV enclosed

WEINGARTEN, SCHURGIN,
GAGNEBIN & LEBOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

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2. I have extensive technical and research experience in the areas of **(please provide your area of expertise to establish that you are a skilled artisan in the field)**. *see CV enclosed*

3. I am one of the inventors of the subject matter described and claimed in the above-identified patent application.

4. I have read and am familiar with the prosecution history of the present application, including the outstanding Office Action dated September 3, 2003 (Paper No. 9).

5. The Detailed Action in the Office Action, starting on page 6, states that claims 1-28 are rejected under 35 U.S.C. §103(a) as being obvious over Nagata et al. (*Natural Toxins* 3:78-86 (1995)) or An et al. (*Toxicon* 32(12):1495-1507 (1994)) in combination with Humphrey et al. (*JACS* 118:11759-11770 (1996)); that claims 1-9 are rejected under 35 U.S.C. §102(b)/103(a) as being unpatentable over each of Nagata et al. or An et al.; and that claims 10-28 are rejected under 35 U.S.C. §103(a) as being obvious over Nagata et al. or An et al. The following addresses distinguishable teachings contemplated in the rejections using the primary references, Nagata et al. and An et al.

6. I have obtained additional experimental data to demonstrate superior properties of the subject matter of the present application in comparison to the antibodies described in Nagana et al. and An et al. It will show that, in particular, there is low variation in cross-reactivity across a very broad range of microcystin congeners as well as excellent quantification

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GAGNEBIN & LEBOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

characteristics when employed in, e.g., a competitive ELISA (*see*, page 24, line 27, to page 25, line 10, and Fig. 5 of the specification).

7. The results of the experiments are graphically presented in the enclosed Fig. 1 (Attachment A). Fig. 1 shows standard curves for the indicated microcystin congeners obtained by a competitive ELISA with an antibody which has been developed against the isolated ADDA group coupled to a carrier as outlined in the Example of the present application (AB#824) (*see*, page 18, line 26, to page 19, line 15, of the specification). The competitive ELISA was performed as described in the present application (*see*, for example, page 19, line 17, to page 20, line 14, of the specification). The results of the measurements as illustrated in Fig. 1 are summarized in the following Table 1.

8. Table 1. Cross-reactivities, limits of quantification (LOQ) and working ranges of ELISA with AB#824.

Congener	I_{80} (Limit of Quantitation) (ng/ml)	Working Range (ng/ml)	Molecular Weight (Da)	I_{50} (50% Inhibition of Binding) (nM)	Cross-reactivity Relative to MC-LR (%)
MC-LR	0.05	0.05-7.50	995.2	0.61	100
MC-RR	0.06	0.06-26.26	1038	1.22	50
MC-YR	0.02	0.02-6.87	1045	0.37	167
MC-LW	0.03	0.03-8.33	1025	0.52	118
MC-LF	0.02	0.02-15.14	986.2	0.57	108
dmMC-LR	0.02	0.02-8.76	981.2	0.39	157
dmMC-RR	0.07	0.07-9.05	1024	0.77	80

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FAX. (617) 451-0313

Nodularin	0.06	0.06-4.5	825	0.61	100

9. The results illustrated in Table I show that when employing the conservative cut-off values of 20% and 80% maximum absorbance, the ELISA had limits-of-quantitation (LOQs) of 0.02-0.07 ng/ml, with working ranges of up to 5-26 ng/ml, depending on the congener. The limit of detection for this assay is one order of magnitude lower (0.002-0.007 ng/ml).

10. Also indicated in Table I, the cross-reactivity among the congeners tested was excellent despite the problems of determining cross-reactivities in the absence of certified reference standards for the congeners. Therefore, at least some of the observed deviation from 100% cross-reactivity can be explained by the facts that (i) all commercially available congener standards contained impurities (guaranteed purity = 95%), and (ii) others have found on re-analysis that the actual quantities of the congener supplied are ranging between ±20% of the quantity stated by the supplier (personal communications to the inventors). Therefore, since cross-reactivities were calculated on the toxin mass specified by the supplier, cross-reactivities between 80 and 120% are most likely not differentiable from 100%.

11. As the difference in the free energy of binding associated with 50% cross-reactivity is very small, only minute conformational or steric interactions of the ADDA moiety could account for the more significant differences in cross-reactivity observed to some congeners. This is, of course, an even greater

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TEL. (617) 542-2290
FAX. (617) 451-0313

problem when the antibody epitope is in a more variable region of the molecule, as is the case for the antibodies described in the prior art which were raised against the complete microcystin toxin conjugated to a protein carrier. This fact explains the great variability of cross-reactivities observed in Nagana et al. and An et al.

12. One of ordinary person skilled in the art would understand that there are fundamental, patentable distinctions between the present application and the cited publications.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the Unites States Code, and that such willful false statements so made may jeopardize the validity of the document, or application, or any patent issuing thereon.

Signed this 4th day of March, 2004.

By: _____
Daniel R. Dietrich

WEINGARTEN, SCHURGIN,
GAGNEBIN & LEBOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313